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Review Articles

Inherited Thrombophilia*: Part 2

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This article continues the review begun in a previous issue of the Journal, which considered the molecular basis and epidemiology of thrombophilia.

Laboratory Investigation of Inherited Thrombophilia

What Laboratory Assays Should be Included?

In order to evaluate the contribution of genetic defects in the pathogenesis of thrombosis, the laboratory investigation should include a functional APC-R test, determination of the factor V genotype and measurements of plasma concentrations of PC, PS (total and free PS) and antithrombin. In addition, a thrombin time will ensure that rare cases of dysfibrinogenemia are not overlooked. At present, available results suggest fibrinolytic parameters not to be of value in the evaluation of individual patients (2).

Before selecting the analytical procedure in the laboratory a number of questions should be addressed such as what are the sensitivity and specificity of the laboratory assay for the genetic defect to be detected? Moreover, the assay performance such as inter- and intra-day variation are important factors to take into account. Quality control should be of high priority. In the choice of assay it is crucial to consider possible influence by other defects, e.g. APC-R influence on coagulation-based functional assays for PC and PS (see below). An important parameter to be taken into consideration is the predictive value of a positive and a negative test. This is determined not only by the specificity and sensitivity of the assay but also by the prevalence in the population to be studied for the particular defect. Thus, unless the specificity is close to 100%, the predictive value of a positive test is usually quite low for defects which are rare in the population. Examples of this will be given below.

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Anticoagulant Protein Deficiencies

A detailed evaluation of the possible contribution of anticoagulant protein deficiencies in the pathogenesis of thrombosis should include analysis of both protein levels with immunological assays and of the functional activity of each of these proteins. However, this is not always possible and practical compromises have to be made. In general, deficiencies of type I (low protein concentration) of any of the anticoagulant proteins are much more common than type II deficiencies (low functional activity but normal protein concentration). An optimal assay for each of these proteins is an easy and cheap functional assay which detects both type I and type II deficiencies. In the case of antithrombin, there are several commercially available functional assays (based on inhibition of either thrombin or factor Xa) which fulfill these criteria. An International Standard for antithrombin is available.

The situation for PC and PS is more complicated because the available coagulation based functional assays are complicated to perform and several of them have low sensitivity and specificity. Assays devised to measure the protein concentrations are often cheaper and easier to perform than several of the commercially available functional assays, and consequently such assays are used in most laboratories in the primary evaluation of the patients. Both sensitivity and specificity of immunological as well as of functional assays are lower when used to test individuals on oral anticoagulation than when used to test patients without anticoagulant therapy.

In the case of PC, depending on the site of the mutation there are many different possible types of functional defects. The interaction with the thrombin-thrombomodulin complex may for instance be impaired with a resulting poor activation; mutations in the vitamin K-dependent region may lead to poor phospholipid binding ability; mutations in other parts of PC may lead to functional defects due to poor interactions with either substrates or cofactors. There is at present no easily available functional assay which monitors all functional aspects of PC and consequently, the laboratory evaluation is a practical compromise. A functional, easy, assay is available for PC and it is used in many laboratories as an alternative to immunological assays in the primary screening of the thrombosis patients. The assay includes activation of PC in plasma with a PC activator (Protac) isolated from a snake venom and measurement of the proteolytic activity of the activated PC against a synthetic substrate. The assay, which is readily automated, is available from several commercial sources. It should be borne in mind that the assay does not detect several of the type II defects. As

there is an overlap in the PC concentrations between normals and those with the genetic defects (3), sensitivity and specificity of all available functional or immunological assays for the genetic defects causing PC deficiency are below 100% The Protac based assays perform quite well and have coefficients of variation below 2% The sensitivity has been estimated to be 85%, whereas the specificity is estimated to be 95% (3) When this assay is used to study a population in which the prevalence is around 0 3%, the predictive value of a positive test is 5% whereas the predictive value of a negative test 99 9% Thus, in screening of the general population this assay is excellent to exclude PC deficiency, whereas a positive test is caused by a genetic defect in only 5% of the cases When the assay is used to test patients with thrombosis (prevalence of genetic defect approximately 3%), the predictive value of a negative test is 34% whereas the predictive value to a positive test is 99 6% The predictive value of a positive test is negatively related to the PC level, i e the lower the PC level the higher the predictive value There is an International Standard for PC available

At present, there are no functional PS assays which fulfill the requirements that can be put on an assay which is to be used in the primary evaluation of thrombosis patients The low specificity of the available functional assays is a major problem as it has recently been shown that functional assays for PS are influenced by the APC-R associated with the presence of the factor V 506Arg to Gln mutation (4) As PS circulates in plasma both as free protein (approximately 30%) and in complex with the C4b-binding protein (5), a relevant question is which fraction of PS should be measured with the immunological assays, the free form, the bound form or total PS The current provisional conclusion is that the free PS assays have higher sensitivity and specificity for the genetic defects causing PS deficiencies than assays for total PS This is because there is a great overlap in the total PS levels between normals and those with the genetic defect (6) Although the prevalence of PS deficiency in the general population is not well established it is reasonable to suspect that it is similar to that of PC and the discussion about the predictive value for a negative and positive test (see above) is therefore relevant also for PS There are several commercially available assays for both free and total PS and an International Standard will become available

The APC-R Test

So far, practical clinical experience has mainly been obtained with the APTT-based test for APC-R (7) In an APTT reaction, the addition of APC results in degradation of both factor VIIIa and factor Va, which delays thrombin generation and hence the formation of the fibrin clot The factor V 506Arg to Gln mutation is associated with partial resistance to degradation of mutated factor Va by APC but the mutated factor Va expresses normal procoagulant properties (8–10) Consequently, thrombin generation is not properly impaired in the presence of APC, which results in less prolongation of the clotting time

As the APC-R test is based on a standard APTT-reaction it is easy to perform The APTT-reaction is run in the presence and absence of a carefully standardised amount of APC, which is included in the calcium chloride solution used to initiate the clotting reaction (7) The clotting time is measured in the presence (a) and absence (b) of APC and a ratio (the APC-ratio) between the two clotting times (a/b) is calculated If the assay is always done on the same instrument and also in other respects performed under strictly standardised conditions, the resulting APC-ratios can be used as they are (11, 12) However, it has been observed that different instruments give different clotting times and APC-ratios obtained on one type of instrument cannot be directly compared with those from another type (13) If results from different laboratories are to be compared, it is beneficial to normalise the APC-ratios against the APC-ratio of a normal plasma pool (14) There are some important considerations to take into account in relation to such a normalisation procedure Due to the high prevalence of APC-R in the general population a pool of normal plasmas is fairly likely to contain plasma from individuals with APC-R and in accordance with this, it has indeed been found that APC-ratios of normal plasma pools are always lower than the mean of the individual APC-ratios For laboratories not having the possibility to perform factor V genotyping, a practical solution to the problem may be to exclude plasmas with extreme APC-ratios from the pool, e g the 5 or 10% with the highest and the 5 or 10% with the lowest APC-ratios In this context, it is obvious that an International Standard plasma would be useful

The handling of the plasma samples is probably of importance and the quality of the results depend on strict standardisation The centrifugation of blood should ensure that the resulting plasma is platelet poor (<1% of normal platelet count), because even a small contamination of platelets affects the APC-ratio, in particular after freezing and thawing of the plasma, platelet contamination will lower the APC-ratio (15) For routine purposes it is recommended to centrifuge the plasma at 2000 g for 20 min at room temperature When the plasma is pipetted off, only the middle portion should preferably be utilised to avoid platelet contamination by native or disrupted platelets

The APC-R test can be performed on both fresh and frozen plasma but "like should be compared with like", i e when fresh plasma is analysed, the normal range should have been determined with fresh plasma, when samples are frozen at –70° C or at –20° C they should be compared with normal controls handled in the same way In support for this concept, it was recently reported that freezing of plasma results in decreased APC-R (Coatest® APC™ Resistance from Chromogenix Molndal, Sweden, was used in the study) but of practical importance the authors found no differences between freezing temperatures (–70° C and –30° C tested) (14) When all variables are strictly controlled, the APC-R test is reliable and gives good discrimination between normal and APC-R individuals The reported sensitivity and specificity of the APC-R test for factor V 506Arg to Gln are 85-95% or better Variation in endogenous levels of PC has of course, no influence on the APC-R because a standardised amount of APC is added In addition, variation of the endogenous free PS level within the range expected for heterozygous PS deficiency and normals has no or only a very minor influence on the APC response in the APTT-based assay (7, 14)

The original APC-R test has a sensitivity of 94% for detection of factor V 506 Gln allele, whereas the specificity is approximately 85% (similar results have been found for the commercial assay from Chromogenix) If this test is used for screening of a population in which the prevalence is around 10%, the negative predictive value is 99 7% whereas the positive predictive value is only 16% In screening a population with thrombosis the negative predictive value is 97% whereas the positive predictive value is around 73% Thus a positive test should always be confirmed by factor V genotyping The sensitivity and specificity of the APC-R test appears to be related to the quality of the APTT reagent

The original APC-R test is not reliable for analysis of plasmas from individuals receiving oral anticoagulants or heparin or if they are derived from individuals with other coagulation defects such as lupus anticoagulants or coagulation factor deficiencies (11 14) In plasma from patients on oral vitamin K antagonists the clotting times in the presence of APC are usually quite long (clotting may not even occur in

such plasmas), which results in high ratios In order to allow analysis of such plasmas, a modified APC-R test, in which sample plasma is prediluted with factor V deficient plasma before assay, was suggested (16-18) A predilution of 1 in 4 of sample plasma with factor V deficient plasma seems to provide sufficient amounts of vitamin K-dependent coagulation factors in the assay to yield normal basal APTT This modification gives valid results not only for patients on oral anticoagulation but has been found also to provide an improved discrimination for the factor V 506 Arg to Gln mutation For some time this modification has been evaluated, using plasmas from individuals with or without oral anticoagulant therapy and a 100% sensitivity and specificity for the presence of the factor V 506 Gln allele was obtained, irrespective of the plasma origin Thus, the modified test is an excellent screening test for the presence of the factor V 506 Gln allele

Analysis for the Factor V 506Arg to Gln Mutation

The codon for Arg506 is positioned close to the exon-intron boundary in exon 10 of the factor V gene (19) Determination of the G to A mutation involves amplification of this nucleotide region either from genomic DNA or from mRNA The detection of the point mutation can be made in many ways, e g by nucleotide sequencing, by different hybridisation techniques, by restriction enzyme cleavage or by allele specific amplification (20-25) The methodology can be optimised to allow analysis of a large number of samples every day The rate limiting step is usually the preparation of patient DNA, even though rather simple extraction procedures can be used It is important to recognise the risk of contamination of PCR-based assays and hence it is of utmost importance to organise the work carefully and include both positive and negative controls

The APC-R Test versus the Factor V Gene Mutation Test

Optimal evaluation of a single patient requires both the functional APC-R test and the factor V gene mutation analysis to be used, because the two methods provide complementary information However, for economical and practical reasons it will not always be possible to do both tests on all individuals Until more experience is obtained by individual investigators it seems that a rational approach would be to perform parallel APC-R tests in the absence and presence of factor V deficient plasma until confidence is obtained with the latter Apart from allowing analysis of plasma from individuals on oral anticoagulant therapy, this approach will also decrease the need for confirmatory genetic testing

When Is it Appropriate to Perform the Laboratory Evaluation?

At present most testing is performed after a thrombotic episode and also after discontinuation of the oral anticoagulation Most of the assays have their highest sensitivity and specificity for the genetic defects if this is strictly followed Testing for PC and PS is not reliable during oral anticoagulation and it is preferable that the patient has been off this kind of therapy for at least 10 days In rare cases it is possible to discontinue the therapy for 10 days during which the patient receives heparin (standard or low molecular weight heparin) A sample can be drawn on the morning of the tenth day before the heparin administration

It will be interesting to evaluate whether prophylactic testing of APC-R before surgery oral contraception, hospitalisation will be beneficial Highly sensitive and specific assays for APC-R with high predictive value are available and inexpensive The main reasons why the other genetic defects are less interesting to evaluate for prophylactic screening are the relatively low levels of sensitivity and specificity which gives low predictive value of a positive test Cost-benefit analysis are required in the evaluation of prophylactic testing

To date it has not been recommended to perform the laboratory investigation in association with the acute thrombotic event mainly because most assays are difficult to interpret and the therapeutic regimes may influence the results of the assays This recommendation may need to be re-evaluated with the availability of the modified highly specific and sensitive APC-R test (with factor V deficient plasma) and factor V genotyping

Where Should the Laboratory Investigation be Performed?

The specialised assays for anticoagulant protein deficiencies are preferably performed in specialised coagulation units whereas it is a matter of debate where the APC-R testing should be done Time will give the APC-R testing its right role in practical medicine and it will at this stage become obvious whether the testing should be performed not only in specialised laboratories but also in major or smaller hospitals

Who Should be Tested?

This question is related to the questions what to test and why It is recommendable that testing is performed on patients with thrombosis and possibly also on their first degree family members It is valid to conclude that there are at present no data to support general screening of the population for APC-R Whether prophylactic APC-R testing is beneficial in association with risk situations is yet to be evaluated

Why Should We Test for Genetic Defects Predisposing for Thrombosis?

For each category of individuals it is important to ask the question why the testing is performed The most pertinent question is whether the results of the assays will affect the handling of the patients This is true for both general screening and for investigation of thrombosis patients The rapid expansion of knowledge in this field will bring new therapeutic recommendations for individuals with genetic predisposition for thrombosis There are two major reasons for testing of family members of patients with thrombosis the prophylactic and diagnostic purposes The prophylactic aspects relate to the possibilities to give adequate advice to family members and the diagnostic purposes to make reliable diagnosis of inherited defects

Clinical Manifestations of Inherited Thrombosis

The most common clinical problem is deep vein thrombosis of the lower limbs, with or without pulmonary embolism, accounting for approximately 90% of all the thrombotic episodes, Table 1 Unusual sites of venous thrombosis, such as those in the mesenteric or cerebral veins, account for less than 5% of the total episodes in patients with antithrombin PC or PS deficiencies in patients with APC-R thrombosis seems to occur less frequently at these sites Superficial thrombophlebitis is more frequent in patients with PC or PS deficiency and APC R than in antithrombin deficient patients Even though a role for PS deficiency as a risk factor for arterial thrombosis has been postulated, there is little current evidence that this or other heterozygous defects of the anticoagulant systems increase the risk of arterial thrombosis (28, 29)

Table I Clinical features of patients with inherited thrombophilia due to defects in the anticoagulant pathways

Venous thromboembolism (>90% of cases)
– Deep vein thrombosis of lower limbs (common)
– Pulmonary embolism (common)
– Superficial thrombophlebitis
– Mesenteric vein thrombosis (rare but characteristic)
– Cerebral vein thrombosis (rare but characteristic)
Family history of thrombosis*
First thrombosis usually at young age (<45 yrs)*
Frequent recurrences*
Neonatal purpura fulminans
(homozygous protein C and protein S deficiency)

* All these features are less evident in patients with APC-R, who appear to be less severely affected clinically

There is history of thrombosis at diagnosis in 50-60% of individuals with antithrombin, PC and PS deficiencies, with a 50% recurrence rate, the first thrombotic episode occurs as early as before 40 years in approximately 80% of patients In antithrombin deficiency, the overall risk of venous thrombosis is considered greater than in PC or PS deficiency (30), but contradictory results have been obtained (29) On the other hand, individuals with APC-R appear to have a lesser tendency to thrombosis than those with the defects of the naturally occurring anticoagulants There is history of thrombosis in only 23% to 31% of cases with APC-R (11, 24) and only 30% of them develop thrombosis before age 45 (31) The presence of APC-R however magnifies the risk of thrombosis in patients with antithrombin, PC and PS deficiencies (32-34), as discussed earlier

In antithrombin, PC and PS deficiencies 32% to 50% of the venous thrombotic episodes occur when other risk factors are concomitantly present (surgery, pregnancy, immobilization) (26-29) In individuals with APC-R, the need of the existence of such risk factors to trigger thrombotic episodes appears to be greater (62%) than for the other thrombophilic syndromes (24)

The risk factors that are more often associated with the occurrence of thrombosis are pregnancy, the puerperium and surgery (24, 26, 27, 29) In women with antithrombin deficiency, the frequency of thrombosis during pregnancy and the puerperium is between 37% and 44%, in PC or PS deficiency, between 12% and 19% (35, 36), in APC-R, 28% (37) Thrombotic episodes occur most frequently during the puerperium, accounting for 60-75% of all the episodes complicating pregnancy (35, 36) A retrospective analysis of a large number of antithrombin, PC or PS deficient individuals gave an overall frequency of venous thrombosis complicating surgery of 22%, with no significant differences due to the type of deficiency or surgical procedure (36) Intake of oral contraceptives is associated with an increased thrombotic risk, particularly in women with antithrombin deficiency and APC-R (38, 39) Patients with dysfunctional defects of antithrombin, PC and PS have thrombotic risks similar to those of the corresponding quantitative defects A notable exception is the antithrombin HBS type II subtype, with a prevalence of thrombosis in these (heterozygous) cases of only 6%, contrasting with 52-68% in patients with other types of antithrombin deficiencies (40)

Homozygous antithrombin deficiency is extremely rare and almost exclusively reported in patients with HBS defects These individuals have a severe thrombotic history of early onset, often affecting arteries (41) Homozygous type I antithrombin deficiency is, presumably, incompatible with life in one report, two brothers with this defect died within three weeks of birth (42) Homozygous PC deficiency has peculiar phenotypic and clinical expressions [reviewed in (43)] In patients with unmeasurable PC, purpura fulminans, due to thrombosis of small vessels with cutaneous and subcutaneous ischemic necrosis, may occur soon after birth or in the first year of life (44, 45) In patients with very low but measurable PC (5-20%), clinical manifestations are milder and generally similar to those for heterozygous deficiency (46) Homozygous PS deficiency has been rarely reported, but is also associated with neonatal purpura fulminans (47) Due to the high frequency of the mutant factor V in the general population, homozygous APC-R is relatively frequent, ~1 5000 (48) Whether homozygotes have a risk of arterial thrombosis greater than that of the general population remains to be elucidated (49, 50)

Management of Inherited Thrombophilia

Acute Events

The management of acute venous thrombosis or pulmonary embolism in patients with inherited thrombophilia is generally not different from other patients Thrombolytic therapy can be used in patients with massive acute venous thrombosis or pulmonary embolism Heparin should be initiated with an intravenous bolus of 5,000 units followed by an infusion of 1400 units per hour (51), or if a weight adjusted regimen is used, a bolus of 80 units per kg body weight followed by an infusion of 18 units per kg per hour (52) The APTT should be performed approximately 6 hours after therapy is initiated and at least daily thereafter to maintain the clotting time in the therapeutic range For many commercial APTT reagents, this corresponds to an APTT that is 1 8 to 3 0 times the mean of the normal range or an anti-factor Xa heparin level of 0 3 to 0 7 units per ml (53) For less sensitive APTT reagents, the therapeutic APPT ratio is 1 5 to 2 0 (54) Warfarin can be started within the first 24 h Heparin is continued for at least 5 days (55) or until the prothrombin time is in the therapeutic range, namely an International Normalized Ratio (INR) of 2 0 to 3 0

Patients with antithrombin deficiency can usually be treated successfully with intravenous heparin (56), though in some situations unusually high doses of the drug are required to achieve adequate anticoagulation In antithrombin deficient patients receiving heparin for the treatment of acute thrombosis, the adjunctive role of antithrombin concentrate purified from human plasma is not clearly defined, as controlled trials have not been performed (56) This product should probably be administered when difficulty is encountered in achieving adequate heparinization, or recurrent thrombosis is observed despite adequate anticoagulation It is also reasonable to treat antithrombin deficient subjects with concentrate before major surgeries or in obstetrical situations where the risks of bleeding from anticoagulation are unacceptable The manufacturing processes used to prepare antithrombin concentrate result in a product that is greater than 95% pure, they also inactivate the hepatitis B and C viruses and human immunodeficiency virus I (57 58) Hence, it is preferable to administer antithrombin concentrate rather than fresh frozen plasma

The infusion of 50 units of antithrombin concentrate per kilogram of body weight (one unit is defined as the amount of antithrombin in one ml of pooled normal human plasma) will usually raise the plasma antithrombin level to approximately 120% in a congenitally deficient individual with a baseline level of 50% (58-62) Plasma levels should be monitored to ensure that they remain above 80%, the administration

of 60% of the initial dose at 24 h intervals is recommended to maintain inhibitor levels in the normal range (62)

Due to the infrequent occurrence of coumarin-induced skin necrosis, it may be advisable to take special precautions when initiating oral anticoagulant treatment in a patient who is previously known or likely to have PC deficiency Warfarin should be started only when the patient is fully heparinized and the dose of the drug should be increased gradually, starting from a relatively low level (e g , 2 mg for the first 3 days and then in increasing amounts of 2 to 3 mg until therapeutic anticoagulation is achieved) Patients with heterozygous PC deficiency and a history of warfarin induced skin necrosis have been successfully retreated with oral anticoagulants Here PC administration either in the form of fresh frozen plasma or PC concentrate provides protection against the development of recurrent skin necrosis until a stable level of anticoagulation is achieved (63, 64)

After an episode of venous thrombosis or pulmonary embolism, patients are usually continued on oral anticoagulants for 3 to 6 months Recent data indicates that the risk of recurrence is greater in patients with permanent as opposed to temporary risk factors for thrombosis (65, 66) and it is therefore appropriate that warfarin should be continued for at least 6 months at an INR of 2 0 to 3 0 in patients with inherited thrombophilia

After 6 months of anticoagulant treatment for an acute thrombotic event, an assessment must be made as to the relative benefit conferred by long-term anticoagulant therapy in preventing future thromboembolic complications versus the potential side-effects, cost, and inconvenience for the patient Unfortunately there is a paucity of reliable data regarding the magnitude of the thrombotic risk or the benefit of anticoagulant treatment in patients with deficiencies of antithrombin, PC, or PS as these are relatively uncommon disorders Due to the high frequency of APC-R in patients presenting with a first episode of venous thrombosis, reliable data is just emerging regarding the risk of recurrence (67) At this time however, only general guidelines (rather than rigid recommendations) for managing patients with the various hereditary defects predisposing to thrombosis will be proposed

Inherited Thrombotic Disorders and Recommendations Pertaining to Duration of Anticoagulant Treatment

When a heterozygous patient with one of the hereditary thrombotic disorders is identified, family studies should be conducted since approximately half of their first-degree relatives will be affected Affected asymptomatic individuals should receive counseling regarding the implications of the diagnosis and advice regarding symptoms that require immediate medical attention In women of child-bearing age, oral contraceptives are generally contraindicated in view of the increased thrombotic risk associated with the use of these medications, although individual circumstances need to be taken into consideration The replacement dose of estrogens that is administered to postmenopausal women is much lower than the contraceptive dose and has not been shown to increase the risk of venous thrombosis in the general population (68) As there are not currently data indicating that these medications increase the risk of thrombosis in patients with a hereditary thrombotic disorder, postmenopausal estrogen replacement is not absolutely contraindicated in women who have a strong indication for replacement therapy

All biochemically affected individuals should be carefully evaluated prior to surgical, medical or obstetric procedures that carry an increased thrombotic risk These subjects should then receive appropriate prophylactic anticoagulation regimens If specific concentrates are available for the patient's deficiency state, under some restricted circumstances these might also be administered to raise the plasma levels of the protein to the normal range during the peri-operative period

In patients with an inherited thrombotic disorder, the occurrence of two or more spontaneously occurring thromboembolic episodes often leads to the continuation of oral anticoagulants for life, even though some feel that the risks of bleeding could exceed those of recurrence of thrombosis

Given that future events in an asymptomatic patient or in an individual with only one prior thrombotic episode cannot currently be accurately predicted and that there is a finite risk of bleeding associated with warfarin therapy, recommendations relating to long-term anticoagulation are best individualized at the current time (69, 70) The clinical features that should be considered in making this decision include

1 Whether the thrombotic episodes were spontaneous or whether precipitating factors were present, e g , if a precipitating event such as a major abdominal operation was present, it would be reasonable to manage the patient without long-term oral anticoagulation after the acute episode was adequately treated
2 The sex and lifestyle of the individual, e g , situations where these factors may influence the decision-making process include women of child-bearing age planning to conceive, occupations that entail prolonged periods of immobilization and thereby might be associated with an increased risk of thromboembolism, jobs with higher than average chance of trauma that might lead to thrombotic or bleeding complications
3 A history of thromboembolism in other biochemically affected members of the family, though marked intra- and inter-familial heterogeneity has been observed in the phenotypic expression of the inherited thrombotic disorders
4 The number, sites, and severity of thrombosis, e g , a patient who previously sustained a massive pulmonary embolus is more likely to receive long-term warfarin than a subject who developed deep venous thrombosis in a calf vein

Management of Pregnancy

The management of pregnancies in women with hereditary thrombotic disorders poses special problems (71) as formal studies evaluating treatments and examining decision analyses are unavailable The incidence of thrombotic complications during pregnancy and the postpartum period appears to be greater in women with antithrombin deficiency than in those with deficiencies of PC or PS (35) Recent data also indicates that 60% of women who develop a first episode of venous thrombosis during pregnancy have a diagnosis of APC-R (10) During pregnancy, adjusted-dose heparin administered by the subcutaneous route is the anticoagulant of choice in many Centres because its efficacy and safety for the fetus are established (72) (Centres with increasing experience of low MW heparins, however, might prefer to use this class of agent, in view of potentially simpler laboratory monitoring) Patients with a history of thrombotic episodes should receive treatment throughout pregnancy, while affected women with antithrombin deficiency who have not yet experienced such events should probably receive treatment Treatment of asymptomatic women with other hereditary thrombotic disorders should be considered on an individual basis

The dose and duration of heparin therapy in pregnancy (and indeed even its overall benefit) is uncertain as appropriately designed clinical trials have not been performed in these patient populations Patients considered to be at high risk should receive full-dose heparin by subcutaneous injection every 12 h for the duration of pregnancy The dose

of heparin should be adjusted to maintain the 6 h postinjection-APTT 1 5 times the control value In women considered to be at intermediate risk, lower doses of heparin can be used (5,000 to 10,000 units subcutaneously every 12 h) and therapy can be started during the second or third trimester and continued for approximately 6 weeks into the postpartum period Low risk patients can be observed closely throughout the pregnancy with duplex ultrasound imaging of the leg veins at regular intervals

In women who are planning pregnancy while chronically taking oral anticoagulants, several approaches can be taken to minimize the risk of both thrombotic complications and warfarin embryopathy One is to stop warfarin and commence subcutaneous heparin therapy this potentially exposes the patient to many months of heparin therapy and the risk of osteoporosis while she is trying to conceive An approach in women with antithrombin deficiency is to use antithrombin concentrates until conception This product, however, is costly and needs to be administered intravenously at frequent intervals Finally, warfarin ther apy could be continued with the performance of pregnancy tests on a frequent basis As soon as pregnancy is diagnosed, and prior to the sixth week of gestation, oral anticoagulants must be discontinued and heparin therapy initiated Though the risk of warfarin embryopathy appears to be quite small during the first six weeks of pregnancy (73), even the small risk of this complication makes this the least preferable of the three approaches

Coumarin-induced Skin Necrosis and Neonatal Purpura fulminans

A clear association has been established between the rare complication of coumarin-induced skin necrosis and hereditary PC deficiency (63, 74) About a third of patients with coumarin-induced skin necrosis will prove to have hereditary PC deficiency (75) This complication has also been described in a patient with heterozygous PS deficiency (76) As coumarin-induced skin necrosis is a rare complication, therapy has been guided primarily by knowledge regarding its pathogenesis The diagnosis should be suspected in patients with painful, red skin lesions developing within a few days after the initiation of the drug and immediate intervention is required to prevent rapid progression and reduce complications Therapy should consist of immediate discontinuation of warfarin, administration of vitamin K, and infusion of heparin at therapeutic doses Lesions, however, have been reported to progress despite adequate anticoagulation with heparin In patients with hereditary PC deficiency, the administration of a source of PC should be seriously considered, and it may also be appropriate in other patients with warfar in-induced skin necrosis as they invariably have reduced plasma levels of functional PC when the skin lesions first appear Fresh frozen plasma has been used, but improved results can be expected with the administration of a highly purified PC concentrate, which facilitates the rapid and complete normalization of plasma PC levels (77)

The management of neonatal purpura fulminans in association with homozygous or doubly heterozygous PC deficiency is more complicated and heparin therapy as well as antiplatelet agents have not been shown to be effective (44, 78-80) The administration of a source of PC appears to be critical in the initial treatment of these patients Fresh frozen plasma has been used with success to treat these infants However, the half-life of PC in the circulation is only about 6 to 12 hours (81, 82), and the administration of plasma on a frequent basis is limited by the development of hyperproteinemia, hypertension loss of venous access, and the potential for exposure to infectious viral agents A highly purified concentrate of PC has been developed and is efficacious in treating neonatal purpura fulminans (83) Warfarin has been administered to these infants without the redevelopment of skin necrosis during the phased withdrawal of fresh frozen plasma infusions (44, 80, 84-86), and this medication has been used chronically to control the thrombotic diathesis A 20 month old child with liver failure and homozygous PC deficiency has undergone successful liver transplantation which normalized his PC levels and resolved the thrombotic diathesis (87) Neonatal purpura fulminans has been described in association with homozygous PS deficiency (88, 89)

Prenatal Diagnosis in Inherited Thrombophilia

Prenatal diagnosis can only be considered appropriate in possible cases of severe thrombophilia where the fetus is expected to be homozygous or compound heterozygous for inhibitor deficiency Only in a few instances has prenatal diagnosis been performed, in attempts to avoid severe thrombophilia The initial procedure used was fetal blood sampling by cordocentesis during the 19th week of gestation in a woman who had previously delivered two newborns who died of massive thrombosis and purpura fulminans due to homozygous PC deficiency (90) At this stage of gestation the mean level of PC antigen is about 10 U/dl (90-92), and thus, care should be taken in distinguishing between results consistent with heterozygosity with a PC level of about 5 U/dl and homozygosity In two reports the mean level of free PS antigen at 15-23 weeks of normal gestation was 27 and 38 U/dl, respectively (91, 92), and hence the diagnosis of homozygous PS deficiency by fetal blood sampling should be easier than the diagnosis of severe PC deficiency Such a procedure has not so far been carried out in cases of suspected severe PS deficiency

A more direct and precise approach to prenatal diagnosis of severe deficiencies of PC, PS and antithrombin depends primarily on the iden tification of the mutation or mutations responsible for the disease in the proband and on devising an easy method for its detection, i e , polymerase chain reaction and restriction analysis or Southern analysis Such work can be performed in highly specialized centers that have a partic ular interest in this issue For identification of the mutation causing PC deficiency a scanning method was devised using denaturant gradient gel electrophoresis (DGGE) of 13 PCR amplified fragments that cover exon I and most of the PC coding regions (93) A similar approach was also used for detection of mutations causing PS deficiency (94) Once these prerequisites have been met, prenatal diagnosis can be provided during the 9-10th week of gestation by chorionic villus sampling and DNA analysis Such an approach was recently used in a family affected by severe PC deficiency (95) and also in a family with antithrombin deficiency type II HBS, in which the fetus was potentially homozygous for a 99 Leu to Phe substitution and heterozygous for factor V 506Arg to Gln (96) Prenatal exclusion of a severe thrombophilia may also be carried out by indirect restriction fragment length polymorphism (RFLP) tracking Such a procedure was used in a family in which a previous infant died of bilateral renal vein thrombosis and both parents were found to be heterozygotes for PS deficiency (97)

Inherited Thrombophilia in the Developing Countries

The prevalence and morbidity of thrombophilia in different geographic regions of the world may be different because of genetic racial differences or because the phenotypic expression of the disease is altered by environmental factors While several studies on inherited thrombophilia have been published, these should be interpreted with caution, due to many problems involved in the execution of the work For example, there are concerns with quality control of laboratory

assays, with objective diagnosis of thrombosis and with study design Furthermore, no large epidemiological studies on groups such as blood donors have been performed in developing countries and there are therefore no reasonable estimates of the prevalence of the inhibitor deficiencies

Despite these reservations, a number of published reports have attempted to address the issues of frequency of venous thrombosis following surgery (98-102) and of hereditary throbophilia (103-105) The available data suggests that the prevalence of deep venous thrombosis may be less in the developing than in the developed countries However, there are suggestions that patients who do get venous thrombosis are more likely to have an underlying inhibitor deficiency Another potentially interesting aspect is that early onset of stroke may be associated with inhibitor deficiency (106) Further work is clearly required to consolidate these various observations

Conclusions

1 At the present time, mutations in four genes are clearly linked to increased risk for venous thromboembolism Many discrete mutations cause deficiencies of antithrombin, PC and PS that diminish the capacity to balance procoagulant activity One specific mutation in factor V 506Arg to Gln has a similar impact by rendering this procoagulant factor resistant to proteolytic degradation With these four established risk factors for thrombosis, roughly 50% of the familial thrombophilias can be explained Apparently a number of other genetic risk factors so far has escaped detection It is however unlikely that these will be found among deficiencies of plasminogen, heparin cofactor II, tissue factor pathways inhibitor or β2 glycoprotein I Other candidates will need further evaluation (for instance, dysfibrinogenemia thrombomodulin defects and inherited hyperhomocysteinemia) It is increasingly apparent that co-inheritance of more than one relatively mild thrombophilic risk factor causes more severe clinical expression

2 Increasingly, attempts are being made to assign a magnitude to genetic and acquired risk factors The risks estimates that have been found in various studies depend heavily on the way the subjects for those studies were selected and do not necessarily apply to individuals selected differently In particular, results from studies among selected families with striking thrombophilia are probably overestimating the risk when applied to individuals who were found because of a single thrombotic event The most stringent selection criteria have been used to ascertain families with the rare abnormalities (PC, PS or antithrombin), which more readily explains the differences found in family studies on these disorders as compared to APC-R than a true difference in severity Finally, when gene-gene and gene-environment interactions are required to bring about thrombosis, there will be differences within families as much as between families

3 Laboratory evaluation of thrombophilia should involve the use of assays with highest possible sensitivity and specificity for the genetic defect that is to be detected Assays can be of immunological and functional nature, in the case of immunological assays it should be realized that cases with truly functional defective proteins may not be detected A practically useful approach should be taken and the selection of analytical procedures should be governed by the aim of the investigation as well as of locally determined factors such as prevalence of the genetic defects to be detected and availability of technical support Based on available scientific information, the laboratory evaluation should include measurements of PC, total and free PS, antithrombin and a functional APC R test which is sensitive and specific for the presence of the factor V 506 Gln allele

In the case of PC, assays which are based on its activation with the PC activator Protac and measurements of the active enzyme with synthetic substrate, fulfil required quality criteria At present no functional PS assays can be recommended for general screening of thrombophilic patients Immunological assays of total as well as of free PS are recommended Recently published results indicate free PS to be the best marker for genetically determined PS deficiency but further studies are required before a recommendation only to measure free PS can be made The functional assays for antithrombin which are based on heparin stimulated inhibition of factor Xa are recommended for screening of thrombophilic patients For initial screening of APC-R, functional tests are recommended The test can be improved by diluting the patient plasma in FV-deficient plasma As assays for PC and PS have distinctly lower sensitivity and specificity for the presence of inherited deficiency during the acute thrombotic epidsode and oral anticoagulation, it is at present recommended to perform the laboratory investigation for these components after discontinuation of the therapy

4 The clinical manifestations of the defects of naturally-occurring anticoagulant systems (antithrombin, PS and PC deficiencies, APC R) are similar In heterozygotes, typical manifestations are those of venous thromboembolism, such as deep-vein thrombosis of the legs, pulmonary embolism and superficial thrombophlebitis Visceral and cerebral vein thrombosis are rarer but quite typical for inherited thrombophilia Patients with homozygous defects have usually more severe clinical manifestations with an earlier age of onset Some manifestations are quite typical for homozygous defects, such as skin necrosis and widespread neonatal thrombosis in PC and PS deficiencies There are preliminary data suggesting that some homozygous defects (antithrombin type II HBS deficiency) may be also associated with an increased risk for arterial thrombosis in the young but more data on this and other homozygous deficiencies are warranted to establish any relationship with arterial disease

5 When a symptomatic patient with inherited thrombophilia due to a known genetic defect is identified family studies should be conducted since approximately half of their first-degree relatives will be affected Asymptomatic individuals who carry the genetic defect should receive counselling regarding the implications of the diagnosis and symptoms that require medical attention In general, the management of symptomatic individuals with the genetic defect is similar to that for symptomatic patients without an identifiable genetic defect An exception is patients with neonatal purpura fulminans in association with homozygous or doubly heterozygous PC deficiency in whom the administration of a source of PC is critical in initial treatment Given that future thrombotic events in patients with inherited thrombophilia cannot be accurately predicted and there is a finite risk of bleeding associated with anticoagulant therapy recommendations relating to long-term treatment are best individualized at the current time

6 Once an individual is defined as being affected by hereditary thrombophilia as many family members as possible are examined for the particular defect detected in the proband and a pedigree is constructed Family members who are found to be affected are counselled about the risk of thrombosis An evaluation of the potential risk of birth of severely affected newborns is usually carried out in families in which intermarriage is practised, and consequently counselling, extensive carrier detection and prenatal diagnosis is planned and executed

The target families for prenatal diagnosis of hereditary thrombophilias are those families that had already been afflicted by infants with severe thrombosis due to homozygosity or compound heterozygosity for PC PS or antithrombin deficiency as well as those families mentioned above In these target families an attempt is made to detect the responsible mutation(s), and to devise a simple method for their

detection, e g , PCR and restriction analysis or Southern analysis This is followed by an extensive study of family members in the child-bearing age for carriership and as a consequence counselling is provided Prenatal diagnosis is then based on DNA obtained by chorionic villus sampling or amniocentesis

In instances in which the mutation cannot be identified, specific RFLP tracking in family members and eventually in fetal DNA is an alternative approach In still other instances of PC or PS deficiency fetal blood sampling at 18-22 weeks of gestation is an option since data on the levels of these components in normal fetuses are available For antithrombin such values are unavailable and thus at present fetal blood sampling for affected families cannot be offered, unless the specific mutation has been identified

7 Deep vein thrombosis and pulmonary embolism have a lower incidence in the developing world when compared to the West, and this may be due to a combination of racial and environmental factors There are limited studies with complete laboratory evaluation on inherited thrombophilia from the developing countries It appears that there is a higher chance of finding an underlying genetic defect (PC PS and anti thrombin deficiency) in patients with thrombosis in the developing world Preliminary data on APC-R suggests that this defect is rare in Asians, Africans and Chinese

Recommendations

1 Recent reports in the literature support the hypothesis that familial thrombophilia is a multiple gene disorder and that the penetrance of the disease is higher in carriers of multiple gene defects In relation to this it is to be expected that prophylactic and therapeutic meas ures need to be adjusted to the number of independent risk factors present in an individual Therefore efforts should be intensified to identify those genetic risk factors that so far have escaped detection, so that these can be included in diagnostic screening procedures
2 Guidelines need to be developed for the use of specific laboratory tests in screening procedures aiming at the identification of individuals who carry a genetic risk factor for venous thrombosis
3 Given the large number of patients that must be followed to detect new abnormalities, collaborative international investigations with standardized recruitment protocols should be encouraged
4 More information linking genetic causes of hyperhomocysteinemia to venous thromboembolism is necessary
5 An estimate of the prevalence of PS deficiency in the population would help evaluate its relative risk
6 More specific recommendations need to be developed for the classification of hereditary PS deficiency Within this context it needs to be established whether so called type III PS deficiency is an independent risk factor for venous thrombosis or a different phenotype of type I PS deficiency
7 As recent data suggest that measurement of free PS may be more valuable than total PS assays for the diagnosis of PS deficiency, it is recommended that studies are performed which evaluate the performance of different methods for determination of free PS
8 General screening of the population for genetic defects of PC, PS and antithrombin can at present not be recommended mainly based on the low prevalence of these defects in the population and on the low predictive value of a positive test It is recommended that studies are performed which address the question whether general screening for APC R (FV 506 Gln allele) e g before oral contraception, surgery or hospitalization is beneficial for the decision on therapeutic and prophylactic regimes
9 Further clinical studies should be organized to evaluate whether defects causing inherited thrombophilia are a cause of or contribute to arterial thrombosis
10 Heterozygous deficiencies of PC, PS or antithrombin should not be the target of prenatal diagnosis In view of the very low expected frequency of the severe homozygous or compound heterozygous defects in PC, PS and antithrombin in the general population it is not recommended to carry out population screening for carriership
11 It would be preferable to establish reference centres where work up of the mutations and DNA based prenatal diagnosis can be performed in cases with severe defects
12 It is necessary to obtain accurate data on the frequency and impact of thrombophilia in the developing world In order to do this individual laboratories should be identified in different regions and these should develop the necessary expertise to screen for and document the genetic defect responsible in association with WHO Collaborating Centres
13 For the present screening should be done on all patients with venous thromboembolism in the context of a study to determine the percentage of patients with hereditary thrombophilia in the developing world where thrombotic disorders in general appear to have a low prevalence Family studies should be done in all patients in whom a genetic defect is documented
14 Since facilities for screening may be available only in reference centers treatment should be initiated without delay where appropri ate and tests performed in the reference center after anticoagulants have been discontinued
15 Data on the thrombotic risk in patients and asymptomatic family members with thrombophilia should be collected to determine whether the risk profile is different in the developing world
16 Careful documentation of the risk of hemorrhage on anticoagulants is necessary in order to determine the risk benefit ratio of therapeutic intervention in patients with thrombophilia
17 Recommendations for screening for hereditary thrombophilia at a national level in health care services can be made only after adequate data on the epidemiology, risk of thrombosis and result of therapeutic intervention is available
18 Since there is a higher incidence of stroke in the young in the developing world this population needs to be studied in order to determine the number of patients who have thrombophilia as the underlying cause
19 In order to increase awareness of inherited thrombophilia in developed and developing countries, it is proposed, in cooperation with the ISTH, to select two centres for designation as WHO Collaborating Centres to improve diagnosis, clinical recognition and treatment of related thrombosis These Centres will serve as reference centres for the appropriate WHO Regions and will improve education of both health professionals and the general public
20 The next meeting of WHO/ISTH experts should be organized at one of the proposed centres for designation as a WHO Collaborating Centre, and focus on the clinical problems of inherited thrombophilia A training course on the diagnosis for thrombophilia for local doctors and invited participants from countries within the WHO Region should also be foreseen at the same time

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